British Journal of Pharmacology (1996) 118, 1367-1370

Effect of treatment with cholecalciferol on the membrane potential and contractility of aortae from spontaneously hypertensive rats

Eneida G. Silva, Lucia M. Vianna, Paulo Okuyama & [1]Therezinha B. Paiva

Department of Biophysics, Escola Paulista de Medicina, Rua Botucatu, 862, 04023-062 São Paulo, SP, Brazil

1 The diet of spontaneously hypertensive rats (SHR) and normotensive Wistar rats (NWR) was supplemented with 12.5 μg cholecalciferol per 100 g body weight daily, by gavage, for 4 weeks.

2 The amplitude of the contractile responses of aortic rings from SHR to potassium and adrenaline, which was smaller than in NWR aortae, was increased after treatment with cholecalciferol. No further changes were observed in the responses of NWR and SHR aortae in the presence of 100 nM apamin.

3 The membrane potentials of aortae from SHR, which were higher than those of aortae from NWR, decreased after treatment with cholecalciferol. Further depolarization was observed in aortic rings from NWR, but not in aortic rings from SHR, after their preincubation with 100 nM apamin.

4 It is concluded that cholecalciferol normalizes the membrane potential and contractility of aortae from SHR, probably through an effect on lipid composition and structure of the plasma membrane.

Keywords: Cholecalciferol; spontaneously hypertensive rats; aorta; membrane potential; contraction

Introduction

Hypertension is characterized by an increased sensitivity of vascular smooth muscle to vasoconstrictor stimuli (Triggle & Laher, 1985; Bohr & Webb, 1988). However, when the vascular reactivity of hypertensive rats was studied, divergent results were observed for different vessels. In general, resistance vessels from spontaneously hypertensive rats (SHR) are more responsive (Aqel *et al.*, 1987; Bendhack *et al.*, 1988; Huzoor-Akbar *et al.*, 1989) whereas in conducting vessels, such as aorta, the contractile responses are lower in SHR than in normotensive animals (Hallbäck *et al.*, 1971; Shibata *et al.*, 1973). In previous work we showed that the amplitude of the contractile responses of aortic rings to potassium is smaller in SHR and in normotensive Wistar-Kyoto rats (WKY) than in normotensive Wistar rats (NWR). The calcium uptake is decreased and the activity of apamin-sensitive Ca^{2+}-dependent K^+ channels is increased in aorta from both WKY and SHR, but an overactivity of the Na^+/K^+-ATPase was observed in aorta from SHR, favouring the activation of Ca^{2+}-sensitive K^+ channels and hyperpolarization of the smooth muscle cell membrane (Silva *et al.*, 1994).

It has been shown that Na^+/K^+-ATPase activity is dependent on membrane fluidity (Kimelberg & Papahadjopoulos, 1974). It is also known that vitamin D_3 affects the lipid structure of the plasma membrane, increasing its fluidity (Rasmussen *et al.*, 1982; Brasitus *et al.*, 1986). Furthermore, SHR present an abnormal vitamin D_3 metabolism (Lucas *et al.*, 1986), and their blood pressure can be reduced by chronic treatment with the vitamin D_3 precursor cholecalciferol (Vianna *et al.*, 1992). Therefore, in the present work, we investigated whether dietary supplementation with cholecalciferol is able to reverse the abnormal membrane potential and contractility of aortae from SHR.

Methods

Animals

Experiments were carried out with an Okamoto-Aoki strain of spontaneously hypertensive rats (SHR) derived from an original colony supplied by the National Institutes of Health, Bethesda, MD, U.S.A. Normotensive Wistar rats (NWR) from the Wistar Institute, Philadelphia, PA, U.S.A., inbred at Escola Paulista de Medicina, SP, Brazil, were also used. Females aged 20-30 weeks were used, which weighed either 160-215 g (NWR) or 170-210 g (SHR). They were fed a standard diet (Labina rat chow, Purina), containing 6,600 i.u. cholecalciferol kg^{-1}, during a basal period of ten days. After the basal period, the treated group received, by gavage, a daily supplementation of 12.5 μg (500 i.u.) cholecalciferol per 100 g body weight, dissolved in 0.35 ml of coconut oil. The duration of the treatment was 4 weeks and a control group received 0.35 ml coconut oil by gavage, daily.

Measurements of mechanical responses

For the aorta tension measurements, the animals were decapitated and bled, the thoracic aorta was removed and placed in Krebs solution (pH 7.40) of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 10. Rings of 1 cm length were cut, everted as previously described (Silva *et al.*, 1994), and the endothelium was removed by scratching with a thin plastic tube wrapped in cotton. For the tension measurements, the preparations were mounted in chambers containing 6.0 ml of Krebs-bicarbonate solution bubbled with a 5% CO_2/95% O_2 mixture and the temperature was kept at 37±0.5°C. After an equilibration period of 2 h under a load of 1 g, the isometric responses were recorded. Cumulative dose-response curves were obtained by stepwise increases in the concentration of KCl or adrenaline until the maximum response was reached, both in the absence and in the presence of 100 nM apamin added to the bath 7 min before the beginning of the experiments.

Measurements of membrane potentials

Membrane potential measurements were made with microelectrodes prepared as previously described (Frediani-Neto *et al.*, 1991; Silva *et al.*, 1994), by pulling capillary glass tubes in a horizontal puller (Narishige, model PN3) and filling with 1 M KCl. The resistance ranged between 30-80 MW. The electrodes were mounted in Ag/AgCl half-cells on a micro-

[1] Author for correspondence.

manipulator (Leitz) and connected to an electrometer (WP Instruments, model FD 223). The signals were recorded in a potentiometric chart recorder (ECB, model RB102).

The impalements were made from the intimal side in everted rings placed in a 2 ml chamber and superfused at a rate of 3 ml min^{-1} with Krebs-bicarbonate solution bubbled with a 5% CO_2/95% O_2 mixture at 37°C. Measurements were performed in preparations that had been previously submitted to a stretching force equal to that which was applied in the tension measurement experiments (1 g for 2 h), both in the absence and in the presence of 100 nM apamin incubated for 7 min before the impalements. The criteria for accepting an impalement were: abrupt changes in the potential recorded upon entry and withdrawal of the microelectrodes from the cell; stable potential (±3 mV) for at least 1 min after impalement; minimal changes in resistance (<10%) of microelectrode after impalement.

Drugs

Vitamin D_3 (cholecalciferol), adrenaline bitartrate and apamin were from Sigma (St. Louis, MO, U.S.A.) and all other chemicals were of the highest purity available.

Statistics

Statistical analysis was done by Student's *t* test for the comparisons between NWR and SHR, or by the Newman-Keuls test for the comparisons between the three different conditions. A probability of $P<0.05$ was considered significant. Values are arithmetic means ± s.e.means.

Results

Mechanical responses

To investigate whether treatment with cholecalciferol could normalize the impaired contractile responses of SHR aortic rings, both SHR and NWR were given 12.5 μg cholecalciferol per 100 g body weight per day by gavage for four weeks. During this period, the animals showed no significant changes in food and water intake or urine output, and had a normal increase of body weight.

Figures 1a and 2a show the cumulative concentration-reponse curves to adrenaline and to hyperosmotic addition of KCl, respectively, in aortic rings from untreated NWR and SHR. The amplitude of the contractile responses to both adrenaline and potassium was significantly greater in aortae from NWR than in those from SHR. After treatment with cholecalciferol (Figures 1b and 2b) a significant increase in the contractile responses was observed in aortic rings from SHR, but no change was seen in those from NWR, so that the amplitude of the contractile responses became similar in the aortae of the two strains.

It has previously been demonstrated that apamin reduces the membrane potential and increases the contractile responses of SHR aortae, suggesting the presence of overactive apamin-sensitive Ca^{2+}-dependent K^+ channels in these arteries (Silva *et al.*, 1994). To determine whether changes in the activity of these channels could account for the normalization of SHR aortic reactivity after vitamin D_3 treatment, the effect of apamin on the concentration-response curves to adrenaline and to KCl was studied in aortic rings from cholecalciferol-treated animals. No further changes in the amplitude of the contractile responses were observed in aortae from treated NWR or SHR after preincubation with 100 nM apamin for 7 min (Figures 1c and 2c).

Membrane potentials

Since electrophysiological properties are also altered in aortae from SHR compared to those from NWR (Silva *et al.*, 1994), in the present work we measured the membrane potentials of aortic rings from cholecalciferol-treated animals. Figure 3 shows that in untreated animals the membrane potentials were significantly higher in aortic rings from SHR than in those of NWR. After treatment with cholecalciferol, a significant depolarization was observed in SHR (Figure 3b) but not in NWR (Figure 3a) aortic preparations. When the membrane potentials were measured in the presence of 100 nM apamin, however, no further changes occurred in the aortic rings from cholecalciferol-treated SHR, whereas a significant depolarization was observed in the corresponding NWR preparations.

Discussion

It is well established that a major physiological effect of 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) is to stimulate the transepithelial transport of calcium across intestinal mucosal cells (Nicolaysen *et al.*, 1952; Haussler & McCain, 1977). Moreover, treatment with vitamin D_3 stimulates calcium uptake in vascular smooth muscle cells (Bukoski *et al.*, 1987; Xue *et al.*, 1991), resulting in increased vascular reactivity (Hatton *et al.*, 1994). Previous studies indicate that the enhanced calcium uptake observed after chronical supplementation with vitamin D_3 is associated with increased synthesis of new pro-

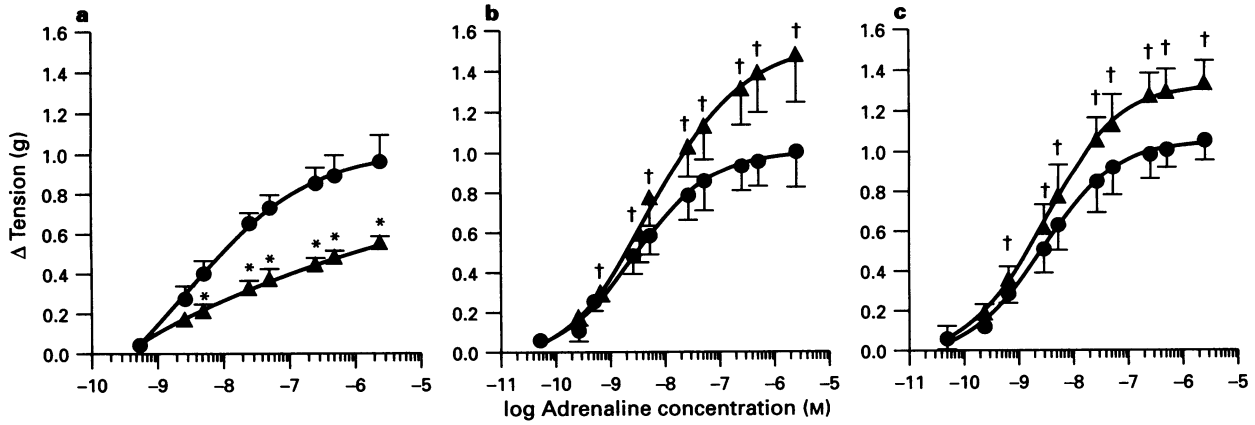

Figure 1 Responses of aortic rings from NWR (●) and SHR (▲) to cumulative increases in adrenaline concentration in control (a) and cholecalciferol-treated animals in the absence (b) and in the presence (c) of apamin (100 nM). Each point is the average of 5–21 experiments and the s.e.means are indicated by vertical lines. *Significantly different from NWR. †Significantly different from control rats, not treated with cholecalciferol ($P<0.05$).

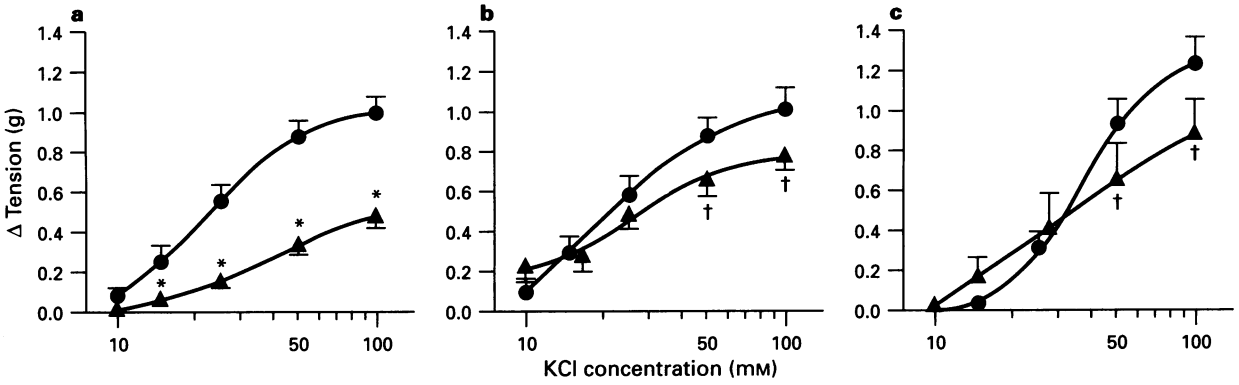

Figure 2 Reponses of aortic rings from NWR (●) and SHR (▲) to cumulative increases in KCl concentration in control (a) and cholecalciferol-treated animals in the absence (b) and in the presence (c) of apamin (100 nM). Each point is the average of 5–21 experiments and the s.e.means are indicated by vertical lines. *Significantly different from NWR. †Significantly different from control rats, not treated with cholecalciferol ($P<0.05$).

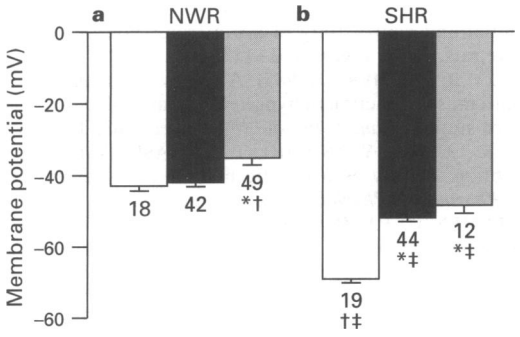

Figure 3 Membrane potentials of aortic rings from NWR (a) and SHR (b) in control (open columns) and cholecalciferol-treated animals in the absence (solid columns) and in the presence (stippled columns) of apamin (100 nM). Values are means of the number of experiments given below the columns, and the s.e.means are indicated by vertical bars. *Significantly different from control animals, not treated with cholecalciferol. †Significantly different from cholecalciferol-treated animals in the absence of apamin. ‡Significantly different from NWR ($P<0.05$).

teins (Haussler & McCain, 1977) and with alterations in lipid composition and structure resulting in an increased plasma membrane fluidity (Matsumoto *et al.*, 1981; Rasmussen *et al.*, 1982; Brasitus *et al.*, 1986). On the other hand, Boland & Boland (1987) showed that the acute administration of 1,25-$(OH)_2D_3$ stimulates calcium uptake in skeletal muscle obtained from vitamin D-deficient chicks, which is abolished by the blockers of voltage-dependent calcium channels, nifedipine and verapamil. These results indicate that vitamin D deprivation, and probably an abnormal vitamin D metabolism, may impaire the calcium movement modulated by lipid fluidity and by the activity of voltage-dependent calcium channels.

Conducting vessels from SHR, such as aorta, show decreased contractility, compared to those of NWR, due to the hyperpolarized state of the plasma membrane and decreased calcium uptake of their smooth muscle cells, probably resulting from an overactivity of the Na^+/K^+-ATPase and of Ca^{2+}-dependent K^+ channels. It has been shown that membrane fluidity can modulate the activity of Na^+/K^+-ATPase (Kimelberg & Papahadjopoulos, 1974) and it is known that vitamin D_3 plays an important role in lipid composition and structure of the plasma membrane, determining an increase in its fluidity (Brasitus *et al.*, 1986). Since SHR present abnormal vitamin D_3 metabolism (Lucas *et al.*, 1986) and reduced fluidity of plasma membrane of erythrocytes and vascular smooth muscles (Tsuda *et al.*, 1992), it is possible that reduced cell membrane fluidity could be the cause of their altered aortic Na^+/K^+-ATPase, Ca^{2+}-sensitive K^+ channels and membrane potential. In accordance with this supposition, it would be expected that treatment with vitamin D_3, by increasing the aortic membrane fluidity, could normalize the activity of the Na^+ pump and of Ca^{2+}-dependent K^+ channels. This could promote membrane depolarization, and an increase of calcium transport modulated by membrane fluidity and/or potential, and would also increase the contractile responses of aortae from SHR.

It is well established that the vascular contraction induced by potassium is mainly the result of an influx of extracellular calcium through voltage-dependent channels (Van Breemen *et al.*, 1972). On the other hand, it was previously demonstrated by Rutledge *et al.* (1984) that α-adrenoceptor agonists mobilize both intra- and extracellular calcium. These authors also showed that noradrenaline induced responses in aortae from SHR were less sensitive to inhibition by calcium antagonists when compared to responses in aortae from normotensive controls. This could indicate that in SHR the contraction induced by α-adrenoceptor agonists results only from intracellular calcium mobilization. In the present work we observed that chronic supplementation with vitamin D did not have an effect on the membrane potential in NWR, but caused a significant decrease in the membrane potential of aortae from SHR. This depolarization in aortae from SHR could restore the normal mobilization of extracellular calcium by both potassium and adrenaline, increasing the responses to these agents. In fact, the aortic contractile responses were affected by the vitamin D_3-treatment in SHR, which increased and became similar to those obtained in NWR. Moreover, these contractile responses, as well as the membrane potential, were not influenced by apamin, similar to those in untreated aortae from NWR (Silva *et al.*, 1994), suggesting that treatment with vitamin D was able to normalize the activity of Ca^{2+}-dependent K^+ channels in the hypertensive strain.

In conclusion, this work shows that chronic oral administration of cholecalciferol normalizes the resting membrane potential and contractility of aortae from SHR.

This work was supported by grants and fellowships from the Brazilian National Research Council (CNP_q) and by the Sao Paulo State Research Foundation (FAPESP). The technical assistance of Nelson A. Mora is gratefully acknowledged.

References

AQEL, M.B., SHARMA, R.V. & BHALA, R.C. (1987). Increased ^{45}Ca influx in response to α1-adrenoceptor stimulation in spontaneously hypertensive rat caudal artery. *J. Cardiovasc. Pharmacol.*, **10,** 205–212.

BENDHACK, L.M., SHARMA, R.V. & BHALLA, R.C. (1988). Contractile response of spontaneously hypertensive rat caudal artery to phorbol esters. *Hypertension*, **11,** I112–I116.

BOHR, D.F. & WEBB, R.C. (1988). Vascular smooth muscle membrane in hypertension. *Ann. Rev. Pharmacol. Toxicol.*, **28,** 389–409.

BOLAND, A.R. & BOLAND, R.L. (1987). Rapid changes in skeletal muscle calcium uptake induced in vitro by 1,25-dihydroxyvitamin D_3 are suppressed by calcium channel blockers. *Endocrinology*, **120,** 1858–1864.

BRASITUS, T.A., DUDEJA, P.K., EBY, B. & LAU, K. (1986). Correction by 1-25 dihydroxycholecalciferol of the abnormal fluidity and lipid composition of enterocyte brush border membranes in vitamin D-deprived rats. *J. Biol. Chem.*, **261,** 16404–16409.

BUKOSKI, R.D., XUE, H. & MCCARRON, D.A. (1987). Effect of $1,25(OH)_2D_3$ and ionized Ca^{2+} on ^{45}Ca uptake by primary cultures of aortic myocytes of spontaneously hypertensive and Wistar Kyoto normotensive rats. *Biochem. Biophys. Res. Commun.*, **146,** 1330–1335.

FREDIANI-NETO, E., SILVA, E.G., FERREIRA, A.T., PAIVA, A.C.M. & PAIVA, T.B. (1991). Increased intracellular Na^+ and depolarization favor angiotensin tachyphylaxis in rabbit aorta. *Am. J. Physiol.*, **260,** H373–H378.

HALLBÄCK, M., LUNDGREN, Y. & WEISS, L. (1971). Reactivity to noreadrenaline of aortic strips and portal vein from spontaneously hypertensive and normotensive rats. *Acta Physiol. Scand.*, **81,** 176–181.

HATTON, D.C., XUE, H., DEMERRITT, J.A. & MCCARRON, D.A. (1994). $1,25(OH)_2$ vitamin D^3-induced alterations in vascular reactivity in the spontaneously hypertensive rat. *Am. J. Med. Sci.*, **307,** S154–S158.

HAUSLLER, M.R. & MCCAIN, T.A. (1977). Basic and clinical concepts related to vitamin D metabolism and action. *N. Engl. J. Med.*, **297,** 1041–1050.

HUZOOR-AKBAR, CHEN, N.Y., FOSSEN, D.V. & WALLACE, D. (1989). Increased vascular contractile sensitivity to serotonin in spontaneously hypertensive rats is linked with increased turnover of phosphoinositide. *Life Sci.*, **45,** 577–583.

KIMELBERG, H.K. & PAPAHADJOPOULOS, D. (1974). Effects of phospholipid acyl chain fluidity, phase transitions, and cholesterol on (Na^+/K^+)-stimulated adenosing triphosphatase. *J. Biol. Chem.*, **249,** 1071–1080.

LUCAS, P.A.A., BROWN, R.C., DRÜEKE, T., LACOUR, B., METZ, J.A. & MCCARRON, D.A. (1986). Abnormal vitamin D metabolism, intestinal calcium transport, and bone calcium status in the spontaneously hypertensive rat compared with its genetic control. *J. Clin. Invest.*, **78,** 221–227.

MATSUMOTO, T., FONTAINE, O. & RASMUSSEN, H. (1981). Effect of 1,25-dihydroxyvitamin D_3 on phospholipid metabolism in chick duodenal mucosal cell. *J. Biol. Chem.*, **256,** 3354–3360.

NICOLAYSEN, R., EEG-LARSEN, N. & MALM, O.J. (1952). Physiology of calcium metabolism. *Physiol. Rev.*, **33,** 424–444.

RASMUSSEN, H., MATSUMOTO, T., FONTAINE, O. & GOODMAN, D.B.P. (1982). Role of changes in membrane lipid structure in the action of 1,25-dihydroxyvitamin D_3. *Fed. Proc.*, **41,** 72–77.

RUTLEDGE, A., SWAMY, V.C. & TRIGGLE, D.J. (1984). Calcium dependence and antagonism of responses to α_1- and α_2-adrenoceptor agonists in vascular tissues from hypertensive and normotensive rats. *Br. J. Pharmacol.*, **83,** 103–111.

SHIBATA, S., KURAHASHI, K. & KUCHII, M. (1973). A possible etiology of contractility impairment of vascular smooth muscle from spontaneously hypertensive rats. *J. Pharmacol. Exp. Ther.*, **185,** 406–417.

SILVA, E.G., FREDIANI-NETO, E., PAIVA, A.C.M. & PAIVA, T.B. (1994). Role of Ca^{2+}-dependent K^+ channels in the membrane potential and contractility of aorta from spontaneously hypertensive rats. *Br. J. Pharmacol.*, **113,** 1022–1028.

TRIGGLE, C.R. & LAHER, I. (1985). A review of changes in vascular smooth muscle function in hypertension: an isolated tissue versus in vitro studies. *Can. J. Physiol. Pharmacol.*, **63,** 355–365.

TSUDA, K., UENO, Y., NISHIO, I. & MASUYAMA, Y. (1992). Membrane fluidity as a genetic marker of hypertension. *Clin. Exp. Pharmacol. Physiol. Suppl.*, **20,** 11–16.

VAN BREEMEN, C., FARINAS, B.R. & GERBA, P. (1972). Excitation-contraction coupling in rabbit aorta studied by the lanthanium method for measuring cellular calcium influx. *Circ. Res.*, **30,** 44–54.

VIANNA, L.M., PAIVA, A.C.M. & PAIVA, T.B. (1992). Treatment with vitamin D_3 reduces blood pressure of spontaneously hypertensive rats. In *Genetic Hypertension.* Vol. 218, ed. Sassard, J., pp. 589–591. London: John Libbey.

XUE, H., MCCARRON, D.A. & BUKOSKI, R.D. (1991). $1,25\ (OH)_2$ vitamin D_3-induced ^{45}Ca uptake in vascular myocytes cultured from spontaneously hypertensive and normotensive rats. *Life Sci.*, **49,** 651–659.

(Received December 5, 1995
Revised March 11, 1996
Accepted March 27, 1996)